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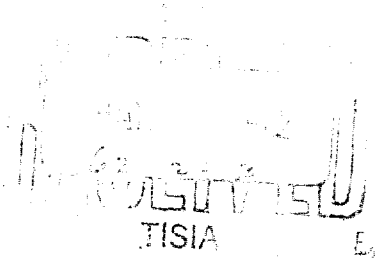

AN IMPROVED MEDIUM FOR PYOCYANIN PRODUCTION BY PSEUDOMONAS AERUGINOSA

The production of pyocyanin by a bacterium from clinical material is considered diagnostic for *Pseudomonas aeruginosa;* however, some isolates of this organism do not exhibit pigment formation. It has even been reported that nonpigmenting strains are the more typical (1). Identification of such apyocyanogenic strains is time-consuming and perhaps somewhat uncertain; it has been maintained that positive identification depends largely upon a history of pyocyanin formation (2). The purpose of this paper is to describe an improved culture medium for rapid identification of *P. aeruginosa* from clinical material by pyocyanin production.

EXPERIMENTAL

Unless otherwise specified, all incubations were at 35° C. for 24 hours. Agar (2 percent) slants containing 1 percent of casein hydrolysate or one of 21 peptones were inoculated in triplicate from 24-hour nutrient agar cultures of 10 stock strains of *P. aeruginosa*. Neopeptone was clearly the best peptone for pigment production; all 10 strains showed pigment on this medium. To determine optimum neopeptone level, concentrations of 0.5, 1.0, 2.0, and 3.0 percent were solidified with 2 percent agar and used as slants. Sabouraud's maltose agar, reportedly the best commercially available medium for pyocyanin stimulation (4, 5, 6), was used for comparative purposes. Two percent neopeptone proved to be optimal and was, in fact, superior to the Sabouraud medium with the majority of 19 *P. aeruginosa* stock strains tested.

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Only a few strains of *P. aeruginosa* produced traces of pyocyanin on plates of 0.4 percent neopeptone agar, although good growth was uniformly observed. The majority of strains produced no visible pigment; therefore, this concentration was used with the impregnated disc technic for assay of substances for enhancement of pyocyanin production and possible use as additives to 2 percent neopeptone agar. No appreciable stimulation occurred with 0.1 molar concentrations of 23 amino acids, nor with 3 concentrations of salts containing potassium, sulfur, phosphorus, magnesium, and iron, despite the fact that these elements have been reported stimulatory for pyocyanin formation in synthetic media (7). When 17 carbohydrates and related compounds were tested at a concentration of 40 percent by the disc technic, pyocyanin production was definitely enhanced by sodium citrate, glycerin, mannitol, fructose, and propylene glycol. Sodium citrate at optimal concentration (distance from the disc) was always superior to any other compound, and glycerin was second best. To determine optimal level of sodium citrate as an additive to 2 percent neopeptone agar, concentrations of 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 percent were incorporated into slants of the neopeptone medium. Tests with 20 stock strains of *P. aeruginosa* showed the 1 percent concentration most effective. Glycerin tested similarly in concentrations of 1, 2, 3, 4, and 5 percent showed no gradation in pyocyanin production. The effect of 1 percent glycerin added to agar containing 2 percent neopeptone and 1 percent citrate was evaluated in coded tubes by three independent observers. There was no observable effect in 24 hours, but after 3 days a slight but definite stimulation of pyocyanin was noted with the majority of the

TABLE I

Comparison of media for pyocyanin production by 20 strains of Pseudomonas aeruginosa *in 15 hours at 35° C.*

Medium	Number of strains showing 0 to 4+ pigment				
	0	1+	2+	3+	4+
Nutrient agar	17	2	1	0	0
Gessard's medium	8	5	6	1	0
Sabouraud's maltose agar	3	3	7	5	2
Neopeptone citrate glycerol agar	1	1	3	2	13

20 strains tested. The final medium, therefore, contained 2 percent neopeptone, 1 percent sodium citrate, 1 percent glycerol, and 2 percent agar. The pH was not adjusted.

Nutrient agar, Gessard's medium (3), Sabouraud's maltose agar, and the neopeptone citrate glycerol agar were compared as to pyocyanin production with 20 stock strains of *P. aeruginosa*. The neopeptone citrate glycerol agar was distinctly superior to any of the other media for pyocyanin production after 15 hours (table 1) and 72 hours. With clinical material, 66 primary isolates suspected of being *P. aeruginosa* all produced pyocyanin on neopeptone citrate glycerol, while 2 failed to form the pigment on Sabouraud's maltose agar. When neopeptone citrate glycerol agar was used in plates for primary isolation, however, young (24 hour) isolated colonies usually were devoid of pyocyanin; similar lack of pigmentation has been observed with all other media for primary isolation.

SUMMARY

For rapid identification of *Pseudomonas aeruginosa* by means of pyocyanin production, a medium has been devised containing 2 percent neopeptone, 1 percent sodium citrate, 1 percent glycerol, and 2 percent agar. With 20 stock strains and 66 primary clinical isolates, this medium appeared superior to media previously employed.

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